

HETEROCYCLES, Vol. 78, No. 1, 2009, pp. 189 - 195. © The Japan Institute of Heterocyclic Chemistry
Received, 19th August, 2008, Accepted, 18th September, 2008, Published online, 22nd September, 2008.
DOI: 10.3987/COM-08-11529

SYNTHESIS OF RELATED SUBSTANCES OF CILOSTAZOL

Jin Zheng,[†] Zheng Liu,[†] Yiru Dai,[‡] Qingjie Zhao,[†] and Jingshan Shen^{*†}

[†] Shanghai Institute of Materia Medica, Chinese Academy of Sciences,
Shanghai 201203, P.R.China

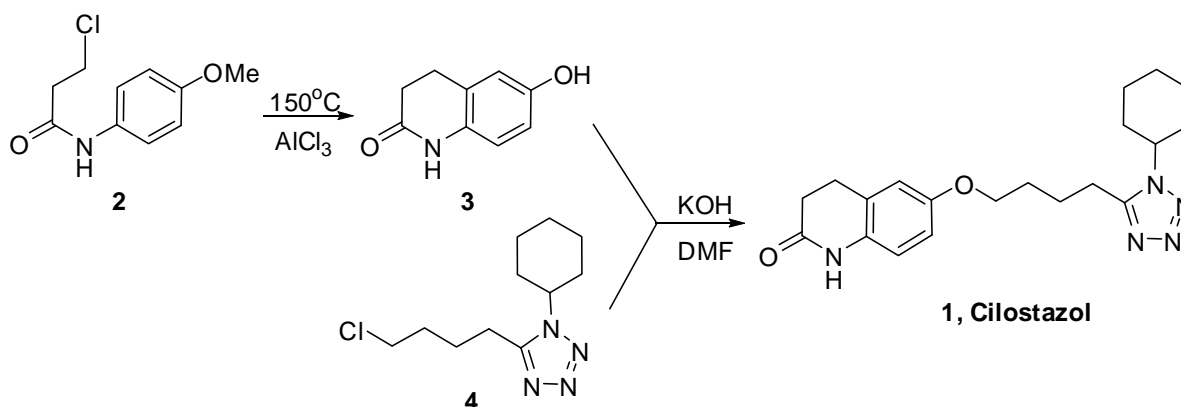
[‡] Topharman Shanghai Co., Ltd., Shanghai 201209, P.R. China

*E-mail: jsshcn@mail.shcnc.ac.cn

Abstract – The impurities in API of cilostazol were detected by LC/MS during the process development. The structures of two impurities **6** and **7** and the related formation mechanisms were proposed. Synthesis of **6** and **7** was conducted for confirmation of the speculated structures.

INTRODUCTION

As a potent and reversible phosphodiesterase III inhibitor, 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone (**1**, cilostazol), is used in clinic for the treatment of intermittent claudication and the control of its impurities is important to clinical safety.¹ The two key steps for the preparation of cilostazol **1** (Scheme 1) involved Friedel-Crafts intramolecular cyclization² of 3-chloro-*N*-(4-methoxyphenyl)propanamide **2** to give the corresponding 3,4-dihydrocarbostyryl **3**, which was then condensed with 5-(4-chlorobutyl)-1-cyclohexyl-1*H*-tetrazole **4** in the presence of potassium hydroxide in DMF to give cilostazol **1**.³ Besides known impurities, we detected two unreported impurities in the final crude product during our process development of cilostazol **1** (Scheme 1).

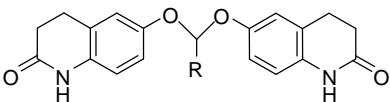
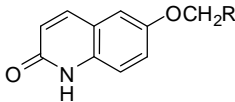
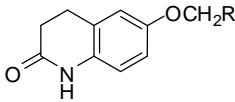
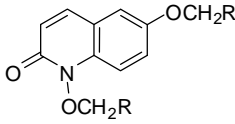
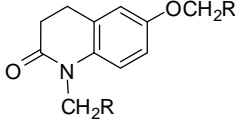


Scheme 1

RESULTS AND DISCUSSION

Four major impurities detected by LC-MS were shown in Table 1. By indication of molecular ion peak and possible formation mechanisms, structures of unknown impurities were suggested. Compound **5**⁵ (M+161) was a known impurity of cilostazol, resulting from the trace amount of 5-(4,4-dichlorobutyl)-1-cyclohexyl-1*H*-tetrazole in the starting material **4**. The unknown impurity **6** (M-2), derived from **9**, was identical to 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1*H*-quinolin-2-one (OPC-13015, the known active metabolite of cilostazol). The impurity **7** (M+220) was formed by the reaction of compound **11** with two equivalents of **4**. Another known impurity **8** (M+206) was formed due to double linking of compound **3** with two equivalents of **4** in the alkaline condition.

Table 1. Structure and LC/MS analysis of cilostazol and its related impurities

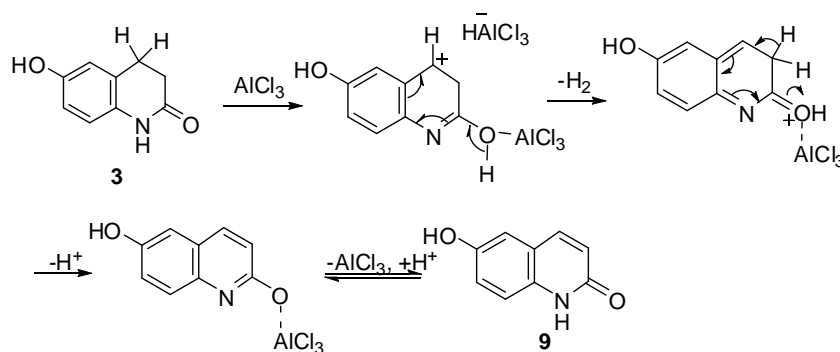
| Compound | RRT | Area (%) | ESI (MH) ⁺ | Structure ^a |
|----------|------|----------|-----------------------|---|
| 5 | 0.85 | 0.12 | 531.4 |  |
| 6 | 0.97 | 0.51 | 368.3 |  |
| 1 | 1.00 | 97.02 | 370.2 |  |
| 7 | 1.53 | 0.35 | 590.3 |  |
| 8 | 1.57 | 0.50 | 576.5 |  |

a) R represents (1-cyclohexyl-1*H*-tetrazol-5-yl)propyl

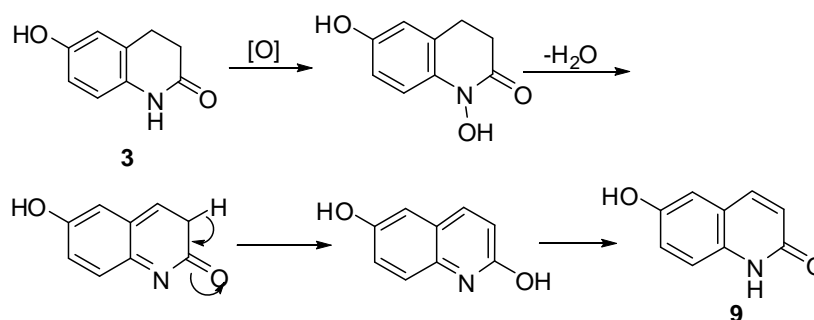
In the Friedel-Crafts reaction of preparing **3**, the possible coordination of the carbonyl with AlCl₃ led to the conversion of the amide group on **3**, from an electron donating substituent into an electron withdrawing group, and reduced the activity of the aromatic ring.⁴ Excess of AlCl₃ (3 equivalents) and high temperature (150 °C) were required to improve the yield of **3**.²

While it remains unclear how **9** was formed during the Friedel-Crafts reaction. Two pathways can be postulated as the formation mechanisms of **9**. Dehydrogenation of 3,4-dicarbostyryl was well-documented in the literatures.⁷ Lewis acids such as AlCl₃ and SbCl₅ sometimes were used as the powerful

dehydrogenating agents.⁸⁻¹⁰ One possible mechanism was AlCl₃-mediated dehydrogenation of **3** (Scheme 2). The other possible mechanism was plausible via the auto-oxidation of **3** at high temperature due to the presence of adventitious O₂ or other oxidants (Scheme 3).

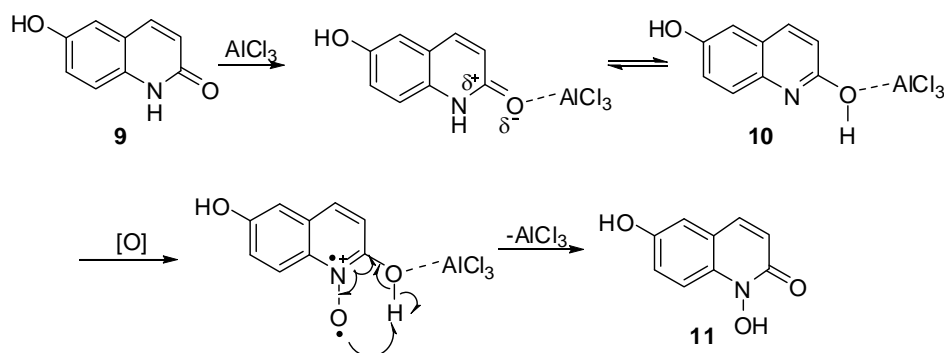


Scheme 2



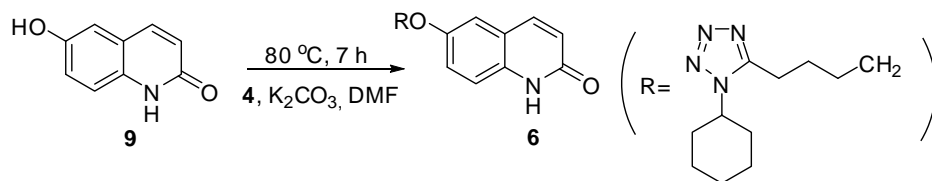
Scheme 3

The LC-MS analysis showed that no *N*-Oxide existed in the starting material of 4-methoxyaniline. The literatures¹¹ indicated that direct oxidation of 2-quinolinone by *m*-CPBA at room temperature led to 1% hydroxamic acid derivative. We postulated that N-O bond of **11** was formed by auto-oxidation of **9**. Coordination of the amide carbonyl with Lewis acid leads to a polarized complex **10**. The polarization facilitated the oxidation on N atom of quinolinol segment, by radical oxygen, to hydroxamic acid **11** (Scheme 4).



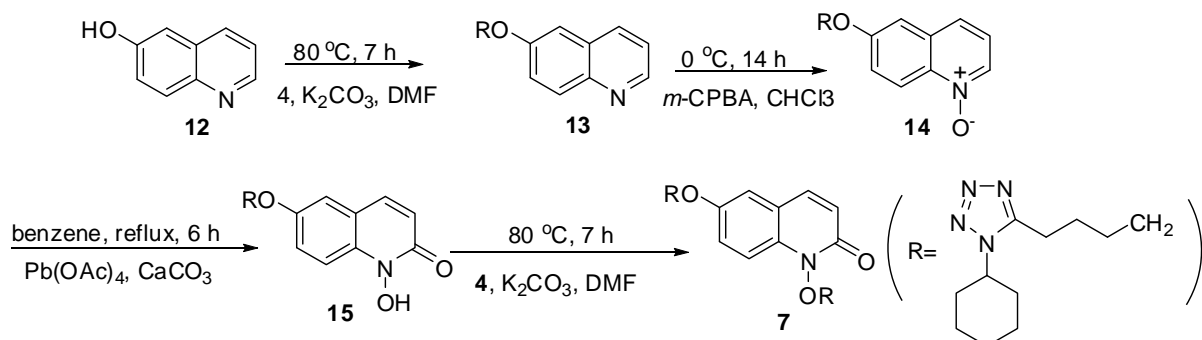
Scheme 4

Based on the above speculation, compounds **6** and **7** were synthesized. Compound **6** was conveniently obtained by reaction of 6-hydroxy-1*H*-quinolin-2-one **9**¹² with **4** (Scheme 5).



Scheme 5

Attempts¹³ to synthesis of hydroxamic acid structure **11** were achieved by oxidation of quinoline, with H₂O₂ in acetic acid, to its *N*-oxide and further oxidation of the resulted *N*-oxide with lead tetraacetate (Scheme 6). Though the oxidation of **12** with H₂O₂¹⁴ in acetic acid led to 6-hydroxyl-1-oxyquinoline in good yield, the following oxidation of the resulted oxyquinoline **14** (R=H) with lead tetraacetate¹⁵ did not give satisfactory result according to the literature procedure, due to the presence of the unprotected hydroxyl group on **14** (R=H). We then modified the strategy, and firstly protected the hydroxyl group on 6-quinolinol by using **4**. The resulted 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]quinoline **13** was oxidized with *m*-CPBA¹⁶ in chloroform at 0 °C to give 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1-oxyquinoline **14** in good yield. 6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1-hydroxyquinolin-2-one **15** was subsequently obtained by oxidation of the oxy-quinoline derivative **14** with lead tetraacetate¹⁵ in presence of CaCO₃ in refluxing benzene. Following the procedure similar to preparation of **6**, the target compound **7** was obtained in 75% yield by the treatment of **15** with **4** in the presence of potassium carbonate.



Scheme 6

Finally, the LC-MS analysis showed that compound **6** and **7** respectively had the same molecular ion peak and relative retention time as the unknown byproducts. Thus, the two unknown byproducts were identical with the speculated structures.

EXPERIMENTAL

Melting points were determined on a WRR melting point apparatus and are uncorrected. NMR spectra were determined using a Mercury 300 MHz, FT NMR spectrometer. Elemental analyses were performed on Elementar Vario EL. Reaction solvents were purchased without further purification. LC-MS analysis was carried out with Perkin-Elmer triple quadrupole mass spectrometer coupled with Agilent 1100 with VWD UV-vis detector. The ion spray voltage of LC-MS spectrum was set at 4.5 kv and temperature was kept at 500 °C and spectra data were acquired from m/z 60 to 800. A YMC C8, 250 mm×4.6 mm 5 μ m particle diameter column, and water-acetonitril (60:40, v: v) and acetonitril as gradient eluents were used for the separation analysis. Detection was carried out at 254 nm and flow rate was maintained at 1.0 mL/min.

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1*H*-quinolin-2-one (**6**)

A mixture of 6-hydroxy-1*H*-quinolin-2-one **9** (207 mmol, 33 g), 5-(4-chlorobutyl)-1-cyclohexyl-1*H*-tetrazole **4** (228 mmol, 55 g) and potassium carbonate (414 mmol, 57 g) in DMF (300 mL) was stirred at 80 °C under nitrogen for 7 h. The reaction mixture was cooled to 25 °C and diluted with water (1.5 L), resulting in the formation of a thick brown precipitate. The precipitate was isolated by filtration, washed with H₂O (3×100 mL), and recrystallized from EtOAc to give **6** (64 g, 85%) as off-white solid, mp 181-183 °C (lit.,¹⁷ 177.5-178.5 °C). ¹H NMR (DMSO-*d*₆): δ 1.14-2.02 (m, 14H), 2.98 (t, 2H, J = 6.3 Hz), 4.04 (t, 2H, J = 6.0 Hz), 4.40 (m, 1H), 6.48 (d, 1H, J = 9.8 Hz), 7.10-7.27 (m, 3H), 7.82 (d, 1H, J = 9.4 Hz), 11.63 (s, 1H). ¹³C NMR (CDCl₃): δ 22.93, 23.82, 24.70, 25.22, 28.36, 32.81, 57.53, 67.56, 109.37, 114.71, 119.52, 120.60, 121.17, 131.49, 136.90, 154.61, 156.94. ESI m/z 368.2 (M⁺ + H). Anal. Calcd for C₂₀H₂₅N₅O₂: C, 65.37; H, 6.86; N, 19.06. Found: C, 65.29; H, 6.99; N, 18.82.

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]quinoline (**13**)

13 was obtained in 80% yield as a white solid by the same procedure as described for **6**, mp 106-108 °C. ¹H NMR (DMSO-*d*₆): δ 1.20-2.16 (m, 14H), 2.95 (t, 2H, J = 7.4 Hz), 4.14 (t, 2H, J = 6.0 Hz), 4.05-4.20 (m, 1H), 7.06 (d, 1H, J = 2.9 Hz), 7.33 (dd, 1H, J_1 = 6.3 Hz, J_2 = 2.9 Hz), 7.36 (dd, 1H, J_1 = 6.3 Hz, J_2 = 4.1 Hz), 8.01 (d, 1H, J = 9.3 Hz), 8.05 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.5 Hz), 8.76 (dd, 1H, J_1 = 4.1 Hz, J_2 = 1.5 Hz). ¹³C NMR (CDCl₃): δ 22.89, 23.86, 24.65, 25.16, 28.27, 32.77, 57.46, 67.24, 105.73, 121.31, 122.22, 129.17, 130.73, 134.71, 144.22, 147.87, 153.40, 156.71. ESI m/z 352.2 (M⁺ + H). Anal. Calcd for C₂₀H₂₅N₅O: C, 68.35; H, 7.17; N, 19.93. Found: C, 68.33; H, 7.18; N, 20.22.

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1-oxyquinoline (**14**)

To a stirred solution of **13** (35.0 g, 0.1 mol) in CHCl₃ (80 mL) was added dropwise a solution of *m*-CPBA

(17.3 g, 0.1 mol) in CHCl_3 (40 mL) at 0 °C for 1.5 h. After further stirred for 4 h, the reaction mixture was washed sequentially with 10% aqueous sodium sulfite, saturated aqueous sodium bicarbonate solution and water. After removal of the chloroform *in vacuo*, the crude product was crystallized from EtOAc to give the pure product **14** (238 g, 65%) as a white solid, mp 175-176 °C. ^1H NMR δ 1.28-2.15 (m, 14H), 2.94 (t, 2H, $J = 7.4$ Hz), 4.14 (t, 2H, $J = 5.8$ Hz), 4.04-4.20 (m, 1H), 7.10 (d, 1H, $J = 2.6$ Hz), 7.25 (dd, 1H, $J_1 = 8.6$ Hz, $J_2 = 6.3$ Hz), 7.34 (dd, 1H, $J_1 = 9.3$ Hz, $J_2 = 2.6$ Hz), 7.62 (d, 1H, $J = 8.6$ Hz), 8.38 (d, 1H, $J = 6.3$ Hz), 8.65 (d, 1H, $J = 9.3$ Hz). ^{13}C NMR (DMSO- d_6): δ 21.80, 23.17, 24.59, 27.79, 32.45, 56.06, 67.61, 107.32, 120.61, 122.34, 122.38, 124.06, 131.86, 133.29, 136.38, 154.08, 158.06. ESI m/z 368.3 ($\text{M}^+ + \text{H}$). Anal. Calcd for $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_2$: C, 65.37; H, 6.86; N, 19.06. Found: C, 65.37; H, 6.89; N, 19.10.

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1-hydroxyquinolin-2-one (**15**)

Lead tetraacetate (15.0 g, wet with acetic acid) and calcium carbonate (0.8 g) were added portionwise to a solution of quinoline *N*-oxide **14** (5.5 g, 1.7 mmol) in benzene (150 mL) at 0 °C for 10 min. The mixture was heated at reflux for 6 h. After cooling, the reaction mixture was filtered to remove inorganic salts. The filtrate was concentrated *in vacuo* to dryness. 3% Dilute HCl (50 mL) was added to the residue, and the mixture heated to 80 °C for 2 h, resulting in the formation of a brown solid. After filtration, the crude product was recrystallized from EtOAc/ CHCl_3 to yield **15** (4.1 g, 72%) as a white solid, mp 135-137 °C. ^1H NMR (CDCl_3): δ 1.22-2.15 (m, 14H), 2.94 (t, 2H, $J = 7.5$ Hz), 4.08 (t, 2H, $J = 6.0$ Hz), 4.13 (m, 1H), 6.84 (d, 1H, $J = 9.3$ Hz), 7.04 (d, 1H, $J = 2.7$ Hz), 7.34 (dd, 1H, $J_1 = 9.3$ Hz, $J_2 = 2.7$ Hz), 7.68 (d, 1H, $J = 9.6$ Hz), 7.76 (d, 1H, $J = 9.6$ Hz). ^{13}C NMR (CDCl_3): δ 22.93, 23.82, 24.70, 25.22, 28.36, 32.81, 57.53, 67.56, 109.37, 114.71, 119.52, 120.60, 121.17, 131.49, 136.90, 154.61, 156.94. ESI m/z 384.3 ($\text{M}^+ + \text{H}$). Anal. Calcd for $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_3$: C, 62.65; H, 6.57; N, 18.26. Found: C, 62.62; H, 6.61; N, 18.31.

1,6-Bis[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1*H*-quinolin-2-one (**7**)

A mixture of 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1-hydroxyquinolin-2-one **15** (4 g 10.4 mmol), 5-(4-chlorobutyl)-1-cyclohexyl-1*H*-tetrazole **4** (2.8 g, 11.5 mmol) and potassium carbonate (2.9 g, 20.8 mmol) in DMF (25 mL) was stirred at 80 °C under nitrogen for 7 h. The reaction mixture was cooled to 25 °C and diluted with water (200 mL), resulting in the formation of a brown precipitate. The precipitate was isolated by filtration, washed with H_2O (3×50 mL), and recrystallized from EtOAc to give **7** (4.6 g, 75%) as a white solid, mp 98-99 °C. ^1H NMR (CDCl_3): δ 1.20-2.23 (m, 28H), 2.93 (t, 2H, $J = 7.5$ Hz), 3.11 (t, 2H, $J = 7.5$ Hz), 4.06 (t, 2H, $J = 6.0$ Hz), 4.13 (m, 1H), 4.29 (t, 2H, $J = 6.0$ Hz), 4.34 (m, 1H), 6.73 (d, 1H, $J = 9.3$ Hz), 7.01 (d, 1H, $J = 2.7$ Hz), 7.19 (dd, 1H, $J_1 = 9.3$ Hz, $J_2 = 2.7$ Hz), 7.47 (d, 1H, $J = 9.6$ Hz), 7.60 (d, 1H, $J = 9.6$ Hz). ^{13}C NMR (DMSO- d_6): δ 21.67, 21.83, 24.59, 26.69, 27.94, 32.46, 32.51, 55.98, 56.06, 67.48, 74.03, 111.15, 112.91, 120.39, 122.66, 132.57, 138.23, 154.09, 156.32. ESI m/z

590.3 ($M^+ + H$). Anal. Calcd for $C_{31}H_{43}N_9O_3$: C, 63.14; H, 7.35; N, 21.38. Found: C, 63.30; H, 7.33; N, 21.61.

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